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(54) Title: MRI CONTRAST AGENT

(57) Abstract

Provision of an MRI contrast agent wherein imaging capability is expressed only within the target abnormal cells, such as tumor, and imaging is not conducted at the site where imaging is not necessary, thereby to strikingly improve the detection sensitivity of the abnormal cells such as tumor. An MRI contrast agent, which comprises a complex of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer, or a complex of a polycationic Gd type contrast agent and an anionic polymer, both complexes being capable of forming a polyion complex, and which expresses an MRI capability at a neutral pH in the presence of a polymer electrolyte.

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MRI CONTRAST AGENT

TECHNICAL FIELD OF THE INVENTION

The present invention relates to an MRI contrast agent. More particularly, the present invention relates to an MRI contrast agent comprising a complex of a polyanionic gadolinium(Gd) type MRI contrast agent and a cationic polymer, or a complex of a polycationic Gd type MRI contrast agent and an anionic polymer.

BACKGROUND OF THE INVENTION

The progress in clinical image diagnosis in recent years is remarkable, and various image diagnoses, such as X ray CT (computed tomography), ultrasonic image diagnosis, MRI (magnetic resonance imaging) diagnosis, scintigraphy and the like, have been used to make a diagnosis of almost every part of the body. Along therewith, various contrast media suitable for such image diagnoses have been developed and found to be useful.

In particular, MRI diagnosis is a new diagnostic method which has been drawing much attention recently from the field of radiation diagnosis as well as entire medical fields. When compared to other contrast agent, the contrast agent for MRI are superior in concentration resolution in tissues and safety from the absence of exposure to X rays, so that they are considered to be clinically useful in locating lesions, grasping anatomical and functional images of normal and abnormal parts, and the like.

On the other hand, the detection capability thereof is not entirely satisfactory, because the detection targets are restricted to certain disease and parts, and leaves room for the development of contrast media having higher functions.

MRI contrast agent have been awaited that (1) permit detection at lower concentrations (small doses), (2) permit detection of specific target cells (e.g., tumor) with high sensitivity, (3) cause no toxicity and (4) are quickly cleared from the body. In particular, the development of MRI contrast medium has been desired, which has superior contrasting capability, which does not form images where imaging is not desired, such as at normal tissues, and which is capable

of forming images only of tumor or specific organs.

Isotope News, July 1998, pp. 7-9, describes an MRI contrast agent recognizing biological microenvironment, wherein it is taught that imaging principles of gadolinium (Gd) type MRI contrast agent are based on shortening of the longitudinal relaxing time (T1) of water molecule by Gd (Lauffer RB, *Chem. Rev.*, 87, 901 (1987)), and that microenvironmental responsive control of interaction between the Gd molecule and water enables on-off switching of the image signals that reflect the microenvironment. Moreover, Mikawa et al., Polymer Preprints, Japan, 46, 2265, 1997 studied variation of T1 relaxing time, that is associated with variation in pH, by making a complex of a cationic polymer and an anionic Gd type contrast agent, based on the report that the pH of tumor tissue is lower than that of normal tissue (Vaupel P. et. al, *Cancer Res.*, 49, 6449 (1989)). This complex forms a strong polyionic complex (PIC) because both of the positive charge and the negative charge become almost equivalent at around a neutral pH, and dehydration of internal water suppresses interaction between the Gd ion and the surrounding water, which inhibits the expression of MRI capability. In contrast, the positive charge is in excess at a weak acidic pH and a strong PIC cannot be formed, whereby the MRI capability is expressed.

SUMMARY OF THE INVENTION

The present invention aims at providing an MRI contrast agent wherein imaging capability is expressed only within the target abnormal cells such as tumor, and imaging is not conducted at the site where imaging is not necessary, thereby to strikingly improve the detection sensitivity of the abnormal cells such as tumor.

The present inventors have conducted intensive studies taking note of the fact that a specific polymer electrolyte expresses on the surface of abnormal cell, such as tumor cell, and found that a contrast agent can be obtained, that is capable of on-off switching of image signal (MRI capability) even at a neutral pH by the presence or absence of this polymer electrolyte, which resulted in the completion of the present invention.

Accordingly, the present invention provides the following.

(1) An MRI contrast agent, which comprises (i) a complex or a gel of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer being capable of forming a polyion complex or a polyion gel, (ii) a complex or a gel of a polycationic gadolinium (Gd) type contrast agent and an anionic polymer being capable of forming a polyion complex or a polyion gel, or (iii) a liposome containing a metal complex which complexes gadolinium (Gd) with a chelating agent being capable of forming a polyion membrane in the liposome; and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte.

(2) The MRI contrast agent of (1) above, which comprises (i) a complex of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer, or (ii) a complex of a polycationic gadolinium (Gd) type contrast agent and an anionic polymer, both complexes being capable of forming a polyion complex, and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte.

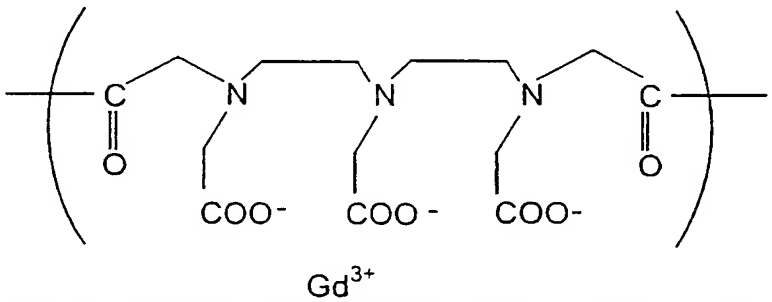
(3) The MRI contrast agent of (1) above which comprises a complex of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer being capable of forming a polyion complex, and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte.

(4) The MRI contrast agent of any of (1) to (3) above, wherein the polyanionic gadolinium (Gd) type contrast agent is (1) a copolymer of (i) a cationic polymer and (ii) a metal complex which complexes gadolinium (Gd) with a chelating agent, and the chelating agent free of gadolinium (Gd) ion, wherein all cations of the cationic polymer are bonded with the metal complex or the chelating agent, or (2) a copolymer of (i) a cationic polymer and (ii) a metal complex which complexes gadolinium (Gd) with a chelating agent.

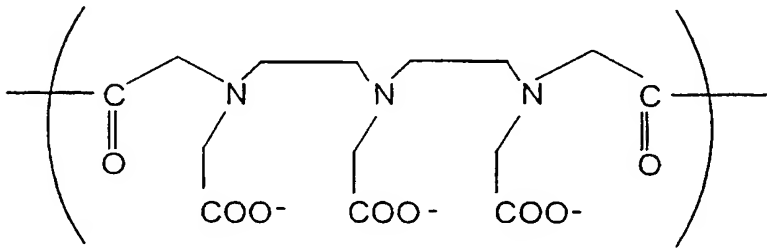
(5) The MRI contrast agent of (4) above, wherein the cationic polymer

copolymerized with the metal complex or chelating agent is a natural polymer chitosan and/or at least one synthetic polymer selected from the group consisting of polydiethylaminoethylmethacrylate (PDEAMA), poly-L-lysine (PLL), poly L-histidine (PLH), poly(vinylamine), poly(ethyleneimine), I,m-ionene, poly(N-alkyl-4-vinylpyridinium chloride) (QPVP), poly(vinylbenzyl trimethylammonium chloride) (PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide].

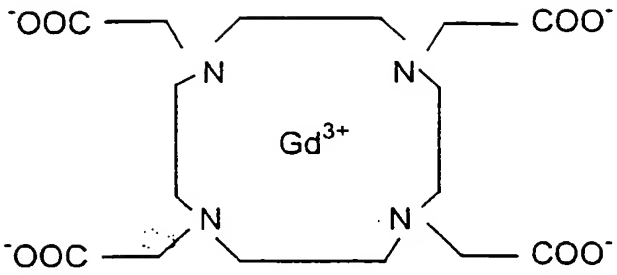
(6) The MRI contrast agent of (4) above, wherein the metal complex is a compound partially having the formula



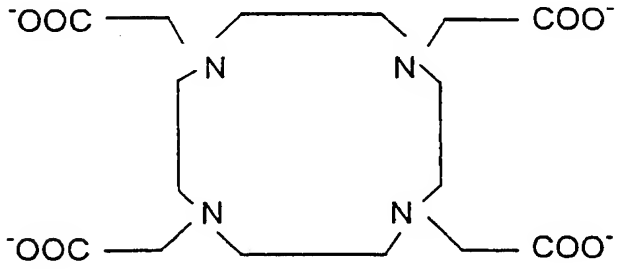
and the chelating agent is a compound partially having the formula



(7) The MRI contrast agent of (4) above wherein the metal complex is a compound partially having the formula,



and the chelating agent is a compound partially having the formula



(8) The MRI contrast agent of any of (4), (6) and (7) above, wherein the cationic polymer is poly L-lysine (PLL).

(9) The MRI contrast agent of (1) above, wherein the polyanionic gadolinium (Gd) type contrast agent is a polymer contrast agent comprising anionic metal complexes polymerized via a spacer molecule.

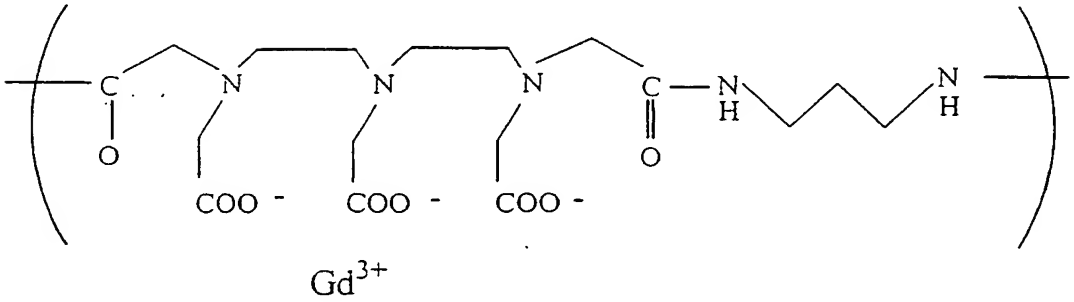
(10) The MRI contrast agent of (9) above, wherein the polymer contrast agent comprising anionic metal complexes polymerized via a spacer molecule is a complex polymer of the formula (1)

$$-(\text{Gd}-\text{DTPA}-\text{PDA})_{x1}-(\text{DTPA}-\text{PDA})_{(100-x1)}- \qquad (1)$$

wherein DTPA is diethylenetriamine pentaacetic acid, PDA is 1,3-propanediamine, x_1 is a real number of 1 to 99, and the formula

$$-(\text{Gd}-\text{DTPA}-\text{PDA})-$$

therein shows a DTPA-PDA moiety into which gadolinium (Gd) has been introduced, namely, the complex polymer represented by the formula

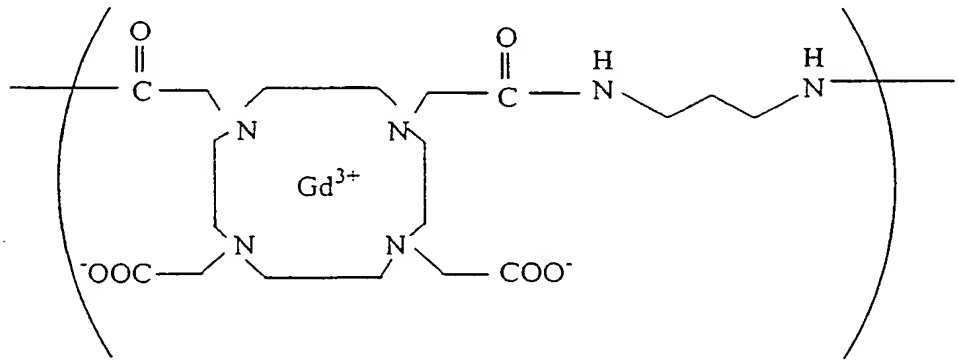


or the formula (2)

(2)

wherein PDA is as defined above, DOTA is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid, x_2 is a real number of 1 to 49, and the formula

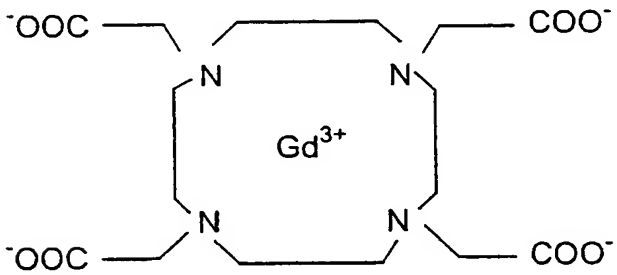
therein shows a DOTA-PDA moiety into which gadolinium (Gd) has been introduced, namely, the complex polymer represented by the formula



(11) The MRI contrast agent of (1) above, wherein the cationic polymer is a natural polymer chitosan and/or at least one synthetic polymer selected from the group consisting of polydiethylaminoethylmethacrylate (PDEAMA), poly L-lysine (PLL), poly L-histidine (PLH), poly(vinylamine), poly(ethyleneimine), l,m-

ionene, poly(N-alkyl-4-vinylpyridinium chloride) (QPVP), poly(vinylbenzyl trimethylammonium chloride) (PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide].

(12) The MRI contrast agent of (1) above, wherein the polycationic gadolinium (Gd) type contrast agent is a bonded compound of a cationic polymer and a metal complex (Gd-DOTA) of one partially having the formula



wherein the metal complex (Gd-DOTA) has bonded to a part of the cation of the cationic polymer and a part of the cation remains unbonded.

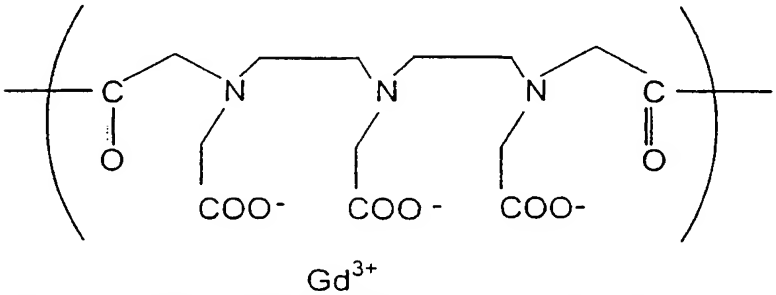
(13) The MRI contrast agent of (12) above, wherein the cationic polymer that bonds to the metal complex (Gd-DOTA) is a natural polymer chitosan and/or at least one synthetic polymer selected from the group consisting of polydiethylaminoethylmethacrylate (PDEAMA), poly L-lysine (PLL), poly L-histidine (PLH), poly(vinylamine), poly(ethyleneimine), I,m-ionene, poly(N-alkyl-4-vinylpyridinium chloride) (QPVP), poly(vinylbenzyl trimethylammonium chloride (PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide].

(14) The MRI contrast agent of (12) above, wherein the cationic polymer is poly L-lysine (PLL) or chitosan.

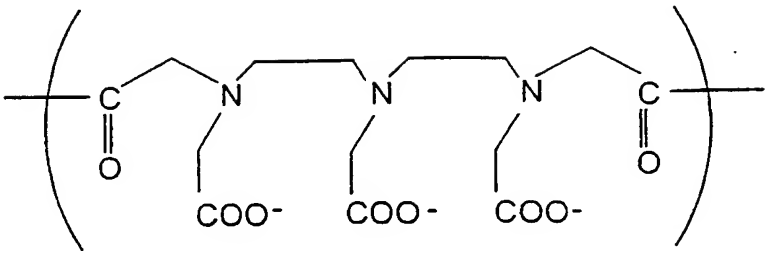
(15) The MRI contrast agent of (1) above, wherein the anionic polymer is at least one member selected from the group of synthetic polymers consisting of poly L-glutamic acid, poly L-aspartic acid, poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly(vinylsulfonic acid), poly(styrenesulfonic acid) (PSS), poly(styrenephosphoric acid) (PSP), polyphosphoric acid, and acidic polysaccharides having colominic acid, sulfonic acid group, carboxylic acid group and/or phosphoric acid group; and the group of natural polymers consisting of hyaluronic acid, chondroitin, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate, and acidic polysaccharides containing sialic Lewis acid, colominic acid, uronic acid, sulfonic acid group, carboxylic acid group and/or a phosphoric acid group.

(16) The MRI contrast agent of (1) above, wherein the polymer electrolyte is at least one member selected from the group consisting of acid glycolipids and glycosaminoglucans.

(17) The MRI contrast agent of (1) above, wherein the polyion complex is a complex of (i) a polyanionic gadolinium (Gd) type contrast agent that is a copolymer of (1) poly L-lysine (PLL) and (2) a metal complex partially having the formula



and a chelating agent partially having the formula



and

(ii) a cationic polymer that is polydiethylaminoethylmethacrylate (PDEAMA).

(18) An MRI contrast agent, which comprises (i) a gel of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer being capable of forming a polyion gel, or (ii) a gel of a polycationic gadolinium (Gd) type contrast agent and an anionic polymer being capable of forming a polyion gel, and which expresses an MRI capability at acidic pH or alkaline pH.

(19) An MRI contrast agent, which comprises a liposome containing a metal complex which complexes gadolinium (Gd) with a chelating agent being capable of forming a polyion membrane in the liposome, and which expresses an MRI capability at acidic pH or alkaline pH.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a chart showing the synthetic process of poly(Gd-DTPA-PDA) (x_1 %).

Fig. 2 shows variation in relative signal intensity due to the administration of the inventive MRI contrast agent in a tumor tissue and muscle.

DETAILED DESCRIPTION OF THE INVENTION

Conventionally, the MRI contrast agent is known to include T1 weighted type contrast agent and T2 weighted type contrast agent. Examples of the T1 weighted type contrast agent include an ionic complex of a chelating agent and gadolinium (Gd), which is a lanthanide metal highly capable of shortening the proton longitudinal relaxing time (T1). The T1 weighted type contrast agent is a positive one which increases brightness of the part where the contrast agent is

present to make the part shine white in an image. The T2 weighted type contrast agent which shortens the transverse proton relaxing time (T2) includes superparamagnetic iron oxide fine particles (magnetite) used in form of a colloid obtained by converting said particles with a dextran derivative. The T2 weighted type contrast agent is a negative one that reduces brightness of the part where the contrast agent is present and makes the part look dark in an image.

The acidic pH in this specification means a pH of from about pH 4 to about pH 6. The alkaline pH in this specification means a pH of from about pH 8 to about pH 9. The neutral pH in this specification means a pH of from about pH 6 to about pH 8.

A complex of a polyanionic Gd type contrast agent and a cationic polymer being capable of forming a polyion complex, or a complex of a polycationic Gd type contrast agent and an anionic polymer being capable of forming a polyion complex generally have near equivalent positive charge and the negative charge at neutral pH (ca. pH 6-8) and form a stable polyion complex, where Gd ion and the surrounding water do not interact and MRI capability is not expressed.

A polyion complex is a collective polymer electrolyte having both the positive and negative charges in the complex.

On the other hand, with regard to the above-mentioned complexes, positive charges become higher corresponding to protonization of polycation usually at acidic pH (ca. pH 4-6), and negative charge decreases corresponding to polyanion transition to non-dissociation state. This has a consequence that positive charge becomes excessive at an acidic pH, thus failing to form a stable polyion complex, where Gd and water interact to express MRI capability.

At an alkaline pH (ca. pH 8-9), positive charges decrease corresponding to deprotonization of polycation, and the negative charge increases corresponding to polyanion transition to dissociation state. Consequently, the negative charge

becomes excessive, again failing to form a stable polyion complex and permitting interaction of Gd and water to express MRI capability.

The complex of a polyanionic Gd type contrast agent and cationic polymer being capable of forming a polyion complex, and the complex of a polycationic Gd type contrast agent and anionic polymer being capable of forming a polyion complex, that constitute the MRI contrast agent of the present invention, generally have nearly the same positive charge and negative charge at a neutral pH, thus forming a strong polyion complex, and its MRI capability is off. However, the positive-negative charge becomes imbalanced in the presence of a polymer electrolyte, and a part or the entirety thereof is dissociated due to the substitution phenomenon of the polyion forming the polyion complex, whereby Gd and its surrounding water interact, and the MRI capability is expressed. When the complexes do not interact with the polymer electrolyte, high Gd ions are concentrated within polyion complex, and MRI signal can be disappeared because of (i) the T2 effect and/or (ii) inhibition of the diffusion of water molecule from inside to outside of polyion complex.

With regard to (i) the gel of a polyanionic Gd type contrast agent and a cationic polymer being capable of forming a polyion gel, and (ii) the gel of polycationic Gd type contrast agent and anionic polymer being capable of forming a polyion gel that constitute the MRI contrast agent of the present inveniton, positive charges become higher corresponding to protonization of polycation and negative charge decreases corresponding to polyanion transition to non-dissociation state at acidic pH (ca. pH 4-6). Consequently, positive charge becomes excessive at an acidic pH (ca. pH 4-6), thus failing to form a strong polyion gel.

At an alkaline pH (ca. pH 8-9), positive charges decrease corresponding to deprotonization of polycation and the negative charge increases corresponding to polyanion transition to dissociation state. Consequently, the negative charge becomes excessive, again failing to form a strong polyion gel and permitting interaction of Gd and water to express MRI capability.

The gels generally have nearly the same positive charge and negative charge at neutral pH, thus forming a strong polyion gel, and its MRI capability is off. However, the positive-negative charge becomes imbalanced in the presence of a polymer electrolyte at a neutral pH, and a part or the entirety thereof is dissociated due to the substitution phenomenon of the polyion forming the polyion gel, whereby Gd and its surrounding water interact, and the MRI capability is expressed. When the gels do not interact with the polymer electrolyte at a neutral pH, high Gd ions are concentrated within polyion gel, and MRI signal can be disappeared because of (i) the T2 effect and/or (ii) inhibition of the diffusion of water molecule from inside to outside of the polyion gel.

With regard to the liposome containing a metal complex which complexes Gd with a chelating agent, being capable of forming a polyion membrane in the liposome that constitute the MRI contrast agent of the present invention, the polyion membrane in the liposome is unstably formed at an acidic pH (ca. 4-6) or alkaline pH (ca. 8-9), whereby activated water molecules surrounding Gd in liposome can diffuse to outside of liposome, and the MRI capability is expressed.

The liposome generally have nearly the same positive charge and negative charge at a neutral pH, thus the polyion membrane in the liposome is stably formed whereby activated water molecules surrounding Gd in liposome can not diffuse to outside of liposome, and its MRI capability is off. However, the positive-negative charge becomes imbalanced in the presence of a polymer electrolyte at a neutral pH, and the polyion membrane in the liposome become unstable whereby activated water molecules surrounding Gd in liposome can diffuse to outside of liposome, and its MRI capability is expressed. When the liposome do not interact with the polymer electrolyte at a neutral pH, high Gd ions are concentrated within the liposome, and MRI signal can be disappeared because of (i) the T2 effect and/or (ii) inhibition of the diffusion of water molecule from inside to outside of the liposome.

Among MRI contrast agents of the present invention, an MRI contrast agent,

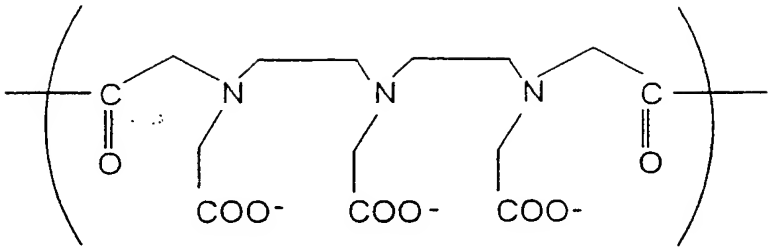
which comprises (i) a complex of a polyanionic Gd type contrast agent and a cationic polymer or (ii) a complex of a polycationic Gd type contrast agent and an anionic polymer, both complexes being capable of forming a polyion complex, and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte, is preferred.

Further, an MRI contrast agent which comprises a complex of a polyanionic Gd type contrast agent and a cationic polymer being capable of forming a polyion complex and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte, is more preferred.

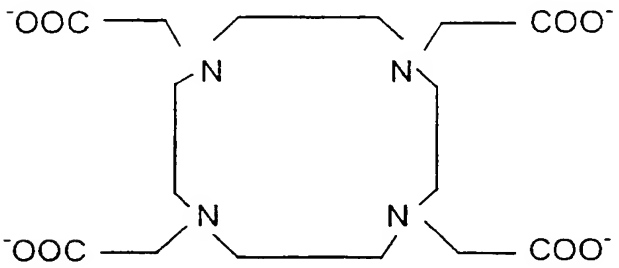
The polyanionic Gd type contrast agent to be used in the present invention is of a T1 weighted type, which is exemplified by (a) a copolymer of (i) a cationic polymer, and (ii) a metal complex which complexes Gd as a Gd ion complex with a chelating agent, and the chelating agent free of Gd ion, wherein all cations of the cationic polymer are bonded with the metal complex or the chelating agent and (b) a polymer contrast agent comprising an anionic metal complex or the chelating agent which has been polymerized via a spacer molecule. Examples of the cationic polymer include synthetic polymers such as polydiethylaminoethylmethacrylate (hereinafter to be abbreviated as PDEAMA), poly L-lysine (hereinafter to be abbreviated as PLL), poly L-histidine (hereinafter to be abbreviated as PLH), poly(vinylamine), poly(ethyleneimine), I,m-ionene, poly(N-alkyl-4-vinylpyridinium chloride) (hereinafter to be abbreviated as QPVP), poly(vinylbenzyl trimethylammonium chloride) (hereinafter to be abbreviated as PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide] and natural polymer such as chitosan. PLL is more preferable in the polymers.

The above-mentioned chelating agent is exemplified by diethylenetriamine pentaacetic acid (hereinafter to be abbreviated as DTPA) partially having the

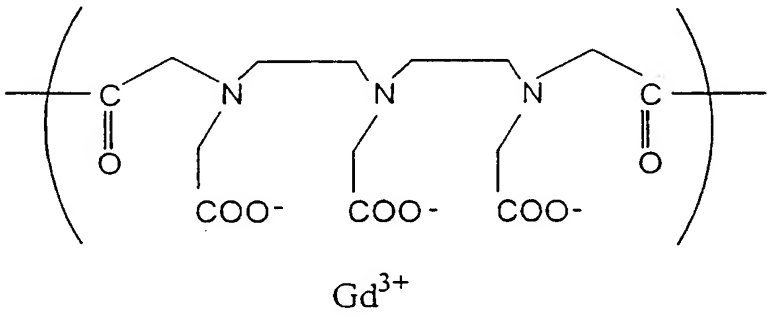
formula



and 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid compounds (hereinafter to be abbreviated as DOTA) partially having the formula

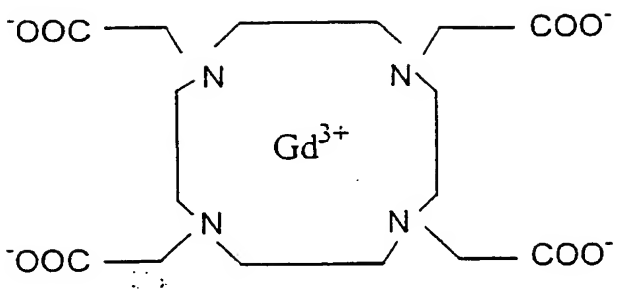


The metal complex which complexes Gd with the chelating agent is exemplified by one partially having the formula



Gd^{3+}

(hereinafter to be abbreviated as Gd-DTPA), and one partially having the formula



(hereinafter to be abbreviated as Gd-DOTA).

With regard to the copolymer of (i) a cationic polymer, and (ii) a metal complex which complexes Gd as a Gd ion complex with a chelating agent, and the chelating agent free of Gd ion, all cations of the cationic polymer are bonded with the metal complex or the chelating agent. The copolymer has a structure expressed by the following formula:

(cationic polymer) – (metal complex) (y%)

wherein y is the proportion of the number of the metal complex to the total number of the metal complex and the chelating agent.

Specific examples of the formula of the copolymer include

(cationic polymer) – poly(Gd-DTPA) (x_3 %)

wherein Gd-DTPA is as defined above and x_3 is a real number of 1 to 99, and

(cationic polymer – poly(Gd-DOTA) (x_4 %)

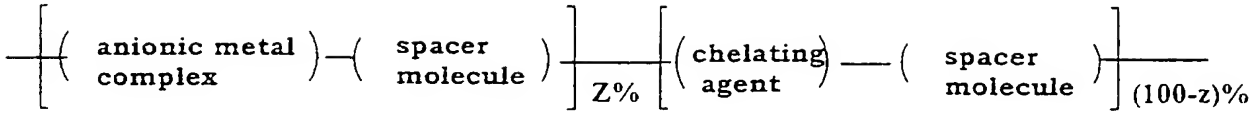
wherein Gd-DOTA is as defined above and x_4 is a real number of 1 to 49.

Preferable example of the copolymer comprising (i) a cationic polymer and (ii) a metal complex which complexes Gd as a Gd ion complex with a chelating agent, and the chelating agent free of Gd ion includes one expressed by the formula:

PLL – poly(Gd-DTPA) (x_3%)

wherein PLL, Gd-DTPA and x_3 are as defined above.

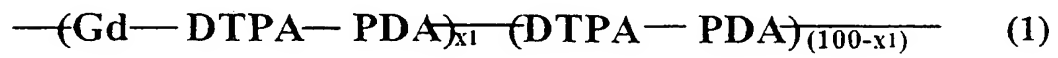
The polymer contrast agent obtained by polymerizing the above-mentioned anionic metal complex via a spacer molecule is represented by the following formula



wherein the anionic metal complex includes Gd-DTPA and Gd-DOTA, the chelating agent is one capable of forming the anionic metal complex, such as DTPA when the anionic metal complex is Gd-DTPA and DOTA when the anionic metal complex is Gd-DOTA, the spacer molecule includes amines such as methylenediamine, ethylenediamine, propanediamine, hexanediamine and the like; active compounds such as neocarzinostatin (NCS) and the like; and the like, and z is a real number that varies depending on the chelating agent.

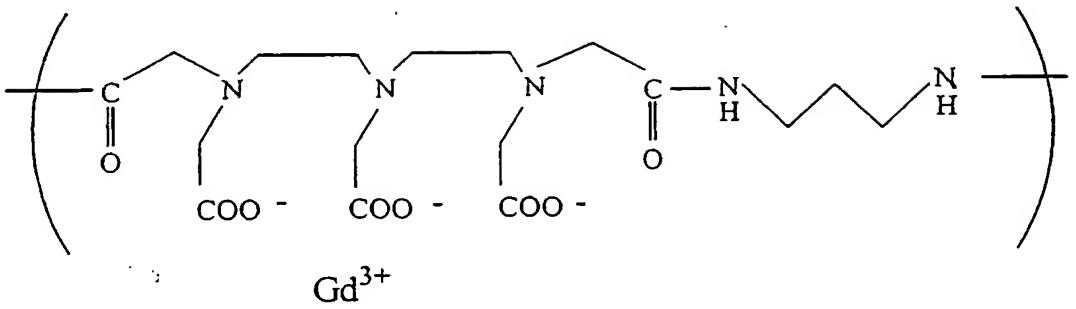
In the above-mentioned spacer molecule, propanediamine is preferable.

As the polymer contrast agents obtained by polymerizing the above-mentioned anionic metal complex via a spacer molecule, specifically (i) the polymer complex of the formula (1)

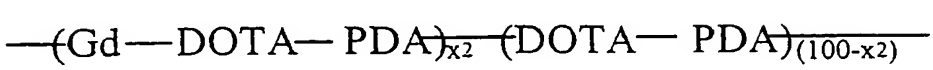


wherein DTPA is diethylenetriamine pentaacetic acid, PDA is 1,3-propanediamine, x_1 is a real number of 1 to 99, and the following formula;

shows the DTPA-PDA moiety into which Gd has been introduced, namely, the formula;



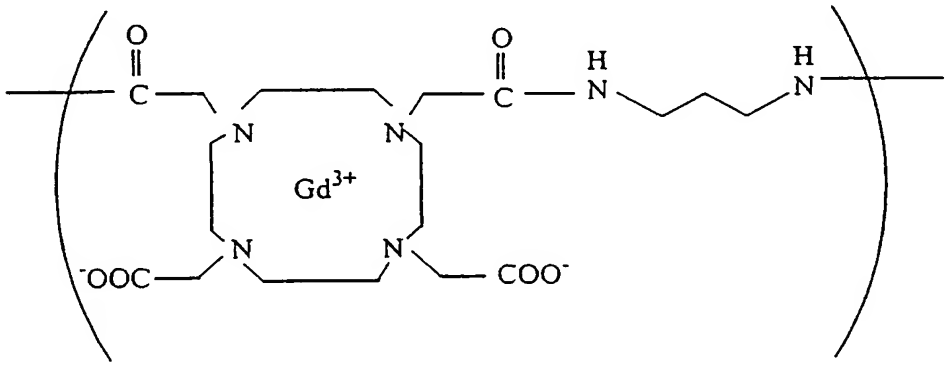
;and (ii) the polymer complex of the formula (2)



(2)

wherein PDA is as defined above, DOTA is 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid, x_2 is a real number of 1 to 49, and the following formula;

shows the DOTA-PDA moiety into which Gd has been introduced, namely, the formula:



; are preferable.

Particularly preferable polymer complex is that of the formula (I).

The formula (1) shows that Gd has been introduced into x_1 % of the DTPA-PDA moiety in the polymer complex, and the polymer complex represented by the formula (1) is hereinafter expressed as poly(Gd-DTPA-PDA)(x_1 %).

Similarly, the formula (2) shows that Gd has been introduced into x_2 % of the DOTA-PDA moiety in the polymer complex. Hereinafter the polymer complex of the formula (2) is expressed as poly(Gd-DOTA-PDA)(x_2 %). The range that x_1 and x_2 can take is as mentioned above.

This anionic metal complex is typically obtained by mixing Gd with a chelating agent in a buffer. The detailed preparation method of poly(Gd-DTPA-PDA)(x_1 %) is described in the following.

The copolymer comprising (i) a cationic polymer, and (ii) a metal complex which complexes Gd as a Gd ion complex with a chelating agent, and the chelating agent free of Gd ion can be typically prepared by mixing by stirring the cationic polymer and the chelating agent in an aqueous solution at room temperature and then introducing Gd into the chelate moiety. For example, polymerization to give the above-mentioned PLL-poly(Gd-DTPA) (x_3 %) wherein x_3 is as defined above can be carried out according to EP No. 0331616 (Example 37), wherein amino group of PLL and one of the five carboxyl groups of DTPA are covalently bonded to synthesize PLL-DTPA and then Gd ion is added.
The polymer contrast agent wherein the above-mentioned anionic metal complex is polymerized via a spacer molecule can be usually prepared by mixing by stirring the spacer molecule and DTPA or DOTA in an aqueous solution at room temperature.

The cationic polymer to be used for forming the complex with a polyanionic Gd type contrast agent for the preparation of a polyion complex in the present invention is exemplified by those mentioned above with regard to cationic polymer used for preparing the above-mentioned polyanionic Gd type contrast agent, with particular preference given to PDEAMA and PLL.

The polycationic Gd type contrast agent to be used in the present invention is, for example, a contrast agent used as a T1 weighted type contrast agent, which is a bonded product of a cationic polymer and a metal complex (Gd-DOTA), wherein the metal complex (Gd-DOTA) is bonded to a part of the cation of the

cationic polymer, e.g., 1-99%, preferably 10-40%, and the rest of the cation remains unbonded.

As the above-mentioned cationic polymer, those used for preparing the above-mentioned polyanionic Gd type contrast agent are similarly used.

As the bonded product of the cationic polymer, which is the above-mentioned polycationic Gd type contrast agent, and Gd-DOTA, a polymer wherein Gd-DOTA has been covalently bonded to a part of the cation of PLL and a polymer wherein Gd-DOTA has been covalent bonded to a part of the cation of chitosan, are preferable.

In this case, Gd-DTPA to be covalently bonded to a part of the cation of cationic polymer is that wherein Gd has been introduced into all (100%) of the DOPA moiety, and DOTA without Gd is not included.

For the preparation of the polycationic Gd type contrast agent, the bonded product of the cationic polymer and the metal complex usually can be prepared by mixing the cationic polymer and the metal complex (Gd-DOTA) in an aqueous solution at room temperature to 40 .

The anionic polymer to be used in the present invention for complexing with a polycationic Gd type contrast agent for the preparation of a polyion complex includes synthetic polymers such as poly L-glutamic acid, poly L-aspartic acid, poly(acryric acid) (hereinafter to be abbreviated as PAA), poly(methacryric acid) (hereinafter to be abbreviated as PMAA), poly(vinylsulfonic acid), poly(styrenesufonic acid) (hereinafter to be abbreviated as PSS), poly(styrenephosphoric acid) (hereinafter to be abbreviated as PSP), polyphosphoric acid, acidic polysaccharides having colominic acid, sulfonic acid group , carboxylic acid group and/or phosphoric acid group and the like, natural polymers such as hyaluronic acid, chondroitin, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate and acid polysaccharides having sialyl Lewis acid, colominic acid, uronic acid, sulfuric acid group carboxylic acid group and/or phosphoric acid group, with preference given to

poly L-glutamic acid and poly L-aspartic acid.

For superior imaging capability, in general terms, MRI contrast agent is preferably polymerized. By the polymerization, molecular rotation rate can be suppressed and the energy of the MRI contrast agent excited by the magnetic field can be easily passed on to the surrounding protons, which in turn shortens the proton relaxing time to increase contrasting capability. Preferably, it has a linear structure showing greater suppressive effect on molecular kinetics. Especially when a complex is formed via 1,3-propanediamine (hereinafter to be abbreviated as PDA) and then polymerized, it forms a linear alternating copolymer structure that is expected to stabilize chelate to a higher degree. For example, the Gd type contrast agent can be polymerized through the polymerization of the metal complex used for preparing the Gd type contrast agent. The polymerization process is shown in Fig. 1.

DTPA anhydride, which is a metal chelating agent, and PDA are polymerized by stirring with heating in DMF (N,N-dimethylformamide) to synthesize poly-DTPA-PDA having a molecular weight of 5,000-30,000, preferably 20,000-30,000, wherein DTPA polymerized linearly via PDA. This synthesized product and Gd are mixed in a buffer to introduce Gd into the DTPA moiety to give a poly(Gd-DTPA-PDA) (x_1 %) which is a polymerized metal complex.

While the proportion of Gd to be introduced into the DTPA-PDA moiety varies depending on the factors such as maintenance of water solubility, charge condition and the like, Gd is introduced into 1-99%, preferably 10-30%, of DTPA-PDA moiety. Hence, x_1 is 1-99, preferably 10-30.

In the present invention, a complex of a polyanionic Gd type contrast agent and a cationic polymer, as well as a complex of a polycationic Gd type contrast agent and an anionic polymer are formed differently depending on the cationic polymer to be used. Generally when PDEAMA is used, for example, the complexes are formed by mixing in an aqueous solution at a pH of around 5-7, preferably around 6.5, at room temperature. Generally when PLH is used, the complexes are formed by mixing in an aqueous solution at a pH of around 4-6,

preferably around 6, at room temperature. Generally when PLL is used, the complexes are formed by mixing in an aqueous solution at a pH of around 7-9, preferably around 8.5, at room temperature.

The ratio in the amounts of polyanionic Gd type contrast agent and cationic polymer, as well as that of polycationic Gd type contrast agent and anionic polymer vary depending on the Gd type contrast agent and polymer to be used and conditions for forming a complex. It is preferable to be prepared so that the charge ratio of polymer : Gd type contrast agent should be set to (0.2 - 5) : 1, more preferably (0.8 - 1.2) : 1.

The MRI contrast agent of the present invention is preferably a complex of (i) a polyanionic Gd type contrast agent consisting of a bonded product of PLL which is a cationic polymer and poly(Gd-DTPA) (x_3 %) wherein x_3 is as defined above, which is a metal complex of Gd ion, and (ii) PDEAMA which is a cationic polymer, and more preferably this complex is one wherein x_3 is 16%, i.e., Gd has been introduced into 16% of the DTPA moiety.

In the present invention, the MRI contrast media of the present invention preferably bonded with a hydrophilic synthetic polymer, polysaccharides and the like. Said synthetic polymer and polysaccharides can be bonded by graft or block copolymerization with the cationic polymer or anionic polymer (main chain polymer), that is used to form a complex with polyanionic Gd type contrast agent or polycationic Gd type contrast agent. Said cationic polymer or anionic polymer, and the above-mentioned synthetic polymer or polysaccharides can be bonded by a conventional method generally used to synthesize graft or block copolymers, though the method varies depending on the cationic polymer or anionic polymer, synthetic polymer or polysaccharides to be used.

Examples of the synthetic polymer suitably used in the present invention include polyethylene, polyethylene glycol, polyoxyethylene glycol, polyethylene terephthalate, polypropylene, polypropylene glycol, polyurethane, polyurethaneurea, pullulonic acid, pullulonic alcohol, polyvinyl polymer, polyvinyl

alcohol, polyvinyl chloride, polyvinylpyrrolidone, nylon, polystylene, polylactate, hydrocarbon fluoride, carbon fluoride, polytetrafluoroethylene, polyacrylate, polyacrylic acid, polymethacrylic acid, polyacrylamide and the like, and their derivatives. In the present invention, those having a molecular weight of about 1,000-100,000, preferably about 5,000-50,000, are used.

Examples of the polysaccharides suitably used in the present invention include arabinan, fluctane, fucan, arabinogalactane, galactane, galacturonan, glucan, mannan, xylane, levan, fucoidan, carrageenan, galactocallolose, pectin, pectinic acid, amylose, plurane, glycogen, amylopectin, cellulose, dextran, pustulan, chitin, agalose, keratin, chondroitin, dermatan, hyaluronic acid, arginic acid, xanthan gum, starch, carboxymethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, methoxycellulose, erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fluctose, sorbose, tagatose, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, neuraminic acid and the like. Those having a molecular weight of about 300-10,000, preferably about 1,000-5,000, are used, without particular limitation on the origin thereof.

The above-mentioned graft or block copolymer has a core-shell structure. This structure conceals Gd in the highly hydrophobic core part, and does not allow Gd to be in contact with the surrounding water molecules, thereby inhibiting image forming. Moreover, since the shell part contains a hydrophilic polymer, it retains water soluble property as a whole. The hydrophilicity suppresses interaction of adsorption of the living body component such as protein in the living body, and taking in to reticuloendothelial system can be avoided, which in turn is expected prolong residence in blood. Moreover, the specific targeting to particular organs and cells by utilizing specific sugar chain recognizing function, is expected.

When this graft or block copolymer is transported to the target site, the balance of the positive charge and negative charge there is lost, thus failing to form a strong polyion complex and then the core-shell structure can not be maintained,

as a result of which it comes into contact with the surrounding water molecule to express imaging capability.

The complex of a polyanionic Gd type contrast agent and a cationic polymer, and the complex of a polycationic Gd type contrast agent and an anionic polymer, that constitute the MRI contrast agent of the present invention, generally have nearly the same positive charge and negative charge at a neutral pH, thus forming a strong polyion complex, and its MRI capability is off. However, the positive-negative charge becomes imbalanced in the presence of a polymer electrolyte, and a part or the entirety thereof is dissociated due to the substitution phenomenon of the polyion forming the polyion complex, whereby the interaction of Gd and water is enabled. The polymer electrolyte producing such an imbalanced positive-negative charge is, for example, sugar chain, glycoprotein and the like having a negative charge in the polymer, inclusive of acid glycolipid having sialyl Lewis acid, colominic acid, uronic acid, sulfuric acid groups and/or phosphoric acid group; glycosaminogycans such as hyaluronic acid, chondroitin, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate and the like; and the like. These substances are known to express in abnormal cells such as various tumor cells and melanoma cells, cells suffering from inflammation and the like. Therefore, on-off switching of MRI capability is possible using tumor cell and the like as a target.

The contrast agent of the present invention can be used in the form of a suspension or solution in a solvent such as distilled water for injection, physiological saline and Ringer solution. Where necessary, a pharmacological acceptable additives such as carrier, excipient and the like can be added. This contrast agent can be applied to cells and the like, and also can be administered to a living body by way of intravascular (vein, artery) administration, oral administration, rectal administration, vaginal administration, lymph duct administration, intraarticular administration and the like. Preferably, it is administered in the form of an aqueous agent, emulsion or suspension. The additives to be used for contrast agent of the present invention vary depending on the mode of administration, administration route and the like.

Specific examples in the case of injection include buffers, antibacterial agents, stabilizers, solubilizers and excipients which are used alone or in combination. In the case of an agent for oral administration, such as aqueous agent, syrup, emulsion and suspension, coloring agent, preservatives, stabilizers, suspending agents, emulsifying agents, thickeners, sweeteners, aromatics and the like are used alone or in combination. Various additives generally used in the pertinent field are used for this end.

The inventive contrast agent for MRI can be administered to form images according to the method used for conventional MRI contrast agent. Specifically, intravenous administration and oral administration can be employed. While the specific dose varies according to the age of administration subjects, the size of body, the parts to be imaged and the like, it is generally 5-100 μmol/kg, preferably 10-50 μmol/kg, in the amount of the Gd type contrast agent contained therein, namely, in the amount of Gd.

The contrast agent of the present invention can be suitably used as a contrast agent for various animals besides human, and the mode of administration, administration route and dose are appropriately determined according to the body weight and conditions of the target animal.

Examples

The present invention is described in more detail in the following by way of Examples and Experimental Examples, to which the present invention is not limited.

Example 1:Preparation of polyanionic Gd type contrast agent - 1 (polymer contrast agent wherein anionic metal complex has been polymerized via spacer molecule)

Synthesis of poly(Gd-DTPA-PDA) (x_1 %)

1) Synthesis of poly(DTPA-PDA)

DTPAA (diethylenetriamine pentaacetic acid anhydride, 12 mM, manufactured by Dojindo) was dissolved in 20 ml of DMF(N,N-dimethylformamide) by heating at 60 . Separately, a solution of PDA (1,3-propanediamine, 12 mM, manufactured by Wako) and TEA (triethylamine, 50 mM, manufactured by Wako) respectively dissolved in 20 ml of DMF was prepared. The both solution were mixed by stirring at 60 for 24 hours. The resulting mixture was evaporated at 80 to solidness and dissolved in about 30 ml of water. This aqueous solution was precipitated with 100% ethanol. The precipitation was collected by filtration and dried to give 5.7 g of crude poly-DTPA-PDA. For further purification, the obtained crude poly-DTPA-PDA was re-dissolved in 30 ml of water and ultrafiltrated (fraction molecular weight: 5000d) to give 0.8 g of purified poly-DTPA-PDA.

2) Preparation of poly(Gd-DTPA-PDA) (x_1 %)

An aqueous solution (1 ml) of 0.1 M gadolinium (Gd) and poly-DTPA-PDA (86.2 mg) obtained in above 1) were mixed in a 0.1 M phosphate buffer (pH 7.2) at room temperature (Gd:DTPA molar rate=1:2) to introduce Gd into DTPA moiety, thereby producing poly(Gd-DTPA-PDA) (50%). In the same manner, Gd and DTPA were mixed at molar rate of Gd:DTPA=1:5 to give poly (Gd-DTPA-PDA) (20%).

Example 2: Preparation of graft copolymer of PLL (cationic polymer) and dextran

PLL HCl salt (100 mg, manufactured by Peptide Institute Inc.) and dextran (100 mg, Mw=2,600, manufactured by Funakoshi) were placed in 15 ml of 0.1 M borate buffer (pH8.5), and 0.3 M sodium cyanoborohydride was added. The mixture was reacted at 45 for 2 days to give PLL-g-dextran (graft rate of dextran 6%).

Example 3: Preparation of graft copolymer of PLL (cationic polymer) and hyaluronic acid

PLL HCl salt (100 mg, manufactured by Peptide Institute Inc.) and hyaluronic acid (100 mg, Mw=8,000, manufactured by DENKI KAGAKU KOGYO KABUSHIKI KAISHA) were placed in 15 ml of 0.1 M borate buffer (pH8.5) and 0.3 M sodium cyanoborohydride and 0.4 M NaCl were added. The mixture was reacted at 37 for 2 days to give PLL-g- hyaluronic acid (graft rate of hyaluronic acid 2%).

Example 4:Preparation of polyion complex - 1
[Preparation of mixed solution of PDEAMA (cationic polymer) and poly(Gd-DTPA-PDA) (20%) (metal complex)]

PDEAMA (polydiethylaminoethylmethacrylate, 4.6 mg) was added to 100 μl of the aqueous solution of poly(Gd-DTPA-PDA) (20%) (20 mMGd/L, 46.24 mg polymer/ml) obtained in Example 1 and mixed (each ingredient has equal charge at this volume proportion). Water was added to make the total amount 1 ml.

Example 5: Preparation of polyion complex - 2
[Preparation of mixed solution of PLH (cationic polymer) and poly(Gd-DTPA-PDA) (20%) (metal complex)]

PLH (poly-L-hystidine, 4 mg, manufactured by Sigma) was added to 100 μl of the aqueous solution of poly(Gd-DTPA-PDA) (20%) (20 mMGd/L; 46.24 mg polymer/ml) obtained in Example 1 and mixed (each ingredient has equal charge at this volume proportion). Water was added to make the total amount 1 ml.

Example 6:Preparation of polyion complex - 3

[Preparation of mixed solution of PLL-g-dextran (graft polymerized cationic polymer) and poly(Gd-DTPA-PDA) (20%) (metal complex)]

PLL-g-dextran (7.25 mg, Mw of dextran=2,600, graft rate of dextran 6%) obtained in Example 2 was added to 100 μl of the aqueous solution of poly(Gd-DTPA-PDA) (20%) (20 mMGd/L, 46.24mg polymer/ml) obtained in Example 1 and mixed (each ingredient has equal charge at this volume proportion). Water was added to make the total amount 1 ml.

Example 7: Preparation of polyion complex - 4

[Preparation of complex of poly(Gd-DTPA-PDA) (20%) (metal complex) and PDEAMA (cationic polymer) in a solution having various pHs (5-9)]

Poly(Gd-DTPA-PDA) (20%) obtained in Example 1 was added to a solution (1 ml, pH 5) so that the Gd concentration was 2 mM. To this solution was added PDEAMA (4.6 mg) and pH was adjusted to a predetermined one (PH 5-9) with a suitable amount of 1N NaOH. Mixing of the same for one hour at room temperature (each ingredient has equal charge at this volume proportion) gave a complex.

Example 8:Preparation of polyion complex - 5

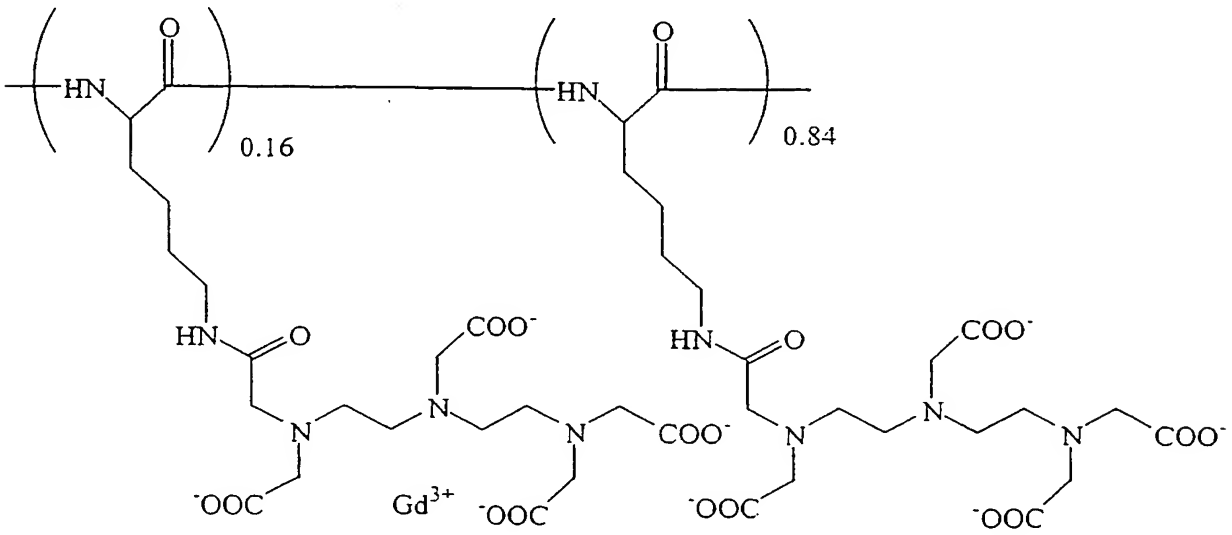
[Preparation of complex of poly(Gd-DTPA-PDA) (20%) (metal complex) and PLH(cationic polymer) in a solution having various pHs (5-9)]

Poly(Gd-DTPA-PDA) (20%) obtained in Example 1 was added to a solution (1 ml, pH 5) so that the Gd concentration was 2 mM. To this solution was added

PLH (4.5 mg) and pH was adjusted to a predetermined one (pH 5-9) with a suitable amount of 1N NaOH. Mixing of the same for one hour at room temperature (each ingredient has equal charge at this volume proportion) gave a complex.

Example 9:Syntesis of polyanionic Gd type contrast agent - 2
Preparation of PLL(Gd-DTPA)(16%)

PLL(Gd-DTPA) which is described in EP 331616 was obtained from Schering AG (Berlin, Germany). In this example, a PLL(Gd-DTPA) (16%) wherein Gd had been introduced into 16% of the DTPA moiety was prepared and used. PLL(Gd-DTPA) was dialyzed against 0.1 M EDTA solution for 4 days and then against water for one week (MWCO=3,500) to produce a polyanion state wherein Gd ions were dissociated. The resulting solution was lyophilized and stored until use. The PLL(Gd-DTPA) (16%) in this state is shown in the following (hereinafter this substance is referred to by the symbol [I]).



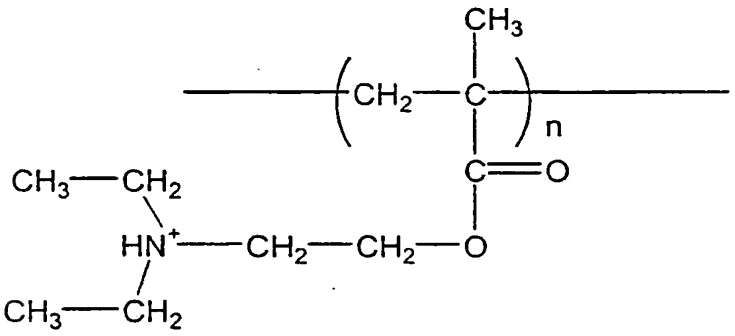
PLL (Gd-DTPA) (16%) [I]

The [Gd]/[DTPA unit] ratio of [I] was confirmed by ICP (inductively coupled plasma, high frequency induction coupled plasma) at a determination

wavelength 342.247 nm, high frequency output 1KW (ICP quanometer, manufactured by Seiko Instruments Inc.) and found to be 0.16. Then, the number average molecular weight of [I] was measured by GPC (gel permeation chromatography). The GPC was conducted using JASCO880-PU pump system under the conditions of flow rate 0.8 ml/min (25) and ultrahydrogel 1000 column (Japan Waters Ltd.). As the mobile phase, an aqueous solution containing 0.5 M acetic acid and 0.3 M sodium sulfate was used. The polymer was detected by refractive index detector (830-RI, JASCO) and multiangle light scattering detector (Dawn-DSP, Wyatt Technology). The number average molecular weight of [I] was 5×10^4.

Example 10: Prepartaion of PDEAMA (cationic polymer)

The poly[2-(diethylamino)ethylmethacrylate] (hereinafter this compound is referred to by the symbol [II]) of the following formula



poly[2-(diethylamino)ethylmethacrylate] [II]

was prepared from the corresponding monomer, DEAMA, by radical polymerization in DMF in the presence of 2,2'-azobis(2,4-dimethylvaleronitrile), which is polymerization initiator, in vacuo at 45 for 3 days.

After polymerization, excess acetonitrile was added to this solution with stirring to allow precipitation of the polymer [II]. This solution was subjected to ultrafiltration using a cellulose triacetate membrane (MWCO=20,000, Sartorius)

to recover polymer [II]. The number average molecular weight of [II] was 8.6×10^4.

Experimental Example 1: MRI signal intensity of contrast agent comprising a complex of PLL(Gd-DTPA) (16%) and PDEAMA at muscle and tumor tissues

The response to pH *in vivo* of the contrast agent of the present invention was confirmed in this experimental example.

(a) Test methods

BALB/c nude mouse (ca. 20 g, female, 8 weeks of age) implanted with colon26 adenocarcinoma cells, which were provided by Assistant Professor Mr. Susumu Nakajima of Asahikawa Medical University, was used as a test animal.

After adjustment of the final Gd concentration to 2.0 mM with 0.15 M NaCl (pH 7), a contrast agent solution (100 μl) containing a mixture of equal amounts of [I] prepared in Example 9 and [II] prepared in Example 10 was injected directly into the tumor site and femoral muscle of this mouse. The MRI imaging and measurement of MRI signal intensity were conducted before injection, immediately after injection and 20 hours after injection. The test solutions were charged in 1 ml disposable syringes (5 mmϕ). The syringes were subjected to MRI imaging using a 4.7 T animal imager (Omega CSI-2, GE-Bruker). The MRI images were obtained by synthesis of T1 and T2-WI (TR/TE=300/12 ms). The MRI imaging and administration of each contrast agent were performed under anesthesia. The results are shown in Fig. 2.

(b) Results

As is evident from Fig. 2, MRI signal intensity at the normal muscle site showed no change even at 20 hours after the administration of the contrast agent and said site was not imaged. At tumor site, however, the signal intensity rose with lapse of time and the tumor tissue was specifically imaged.

The mechanism to exert such tissue specific imaging capability is considered to be attributable to the imaging capability of the contrast agent of the present invention which is in the on-state due to the dissociation of the polyion complex because of the imbalance of positive-negative charge of contrast agent caused by the sugar chain (e.g., sialyl Lewis acid) expressed on the cell surface of colon26 cells.

Experimental Example 2:

When colominic acid, which is an acidic polysaccharide, is added at pH 7 to a polyion complex of PLL(Gd-DTPA) (16%) and PDEAMA, the MRI capability (R1 relaxivity) increases in a dose dependent manner.

Experimental Example 3:

With regard to a polyion complex of poly(Gd-DTPA-PDA) (20%) and PDEAMA, when the mixing ratio of poly(Gd-DTPA-PDA) (20%) and PDEAMA is varied in terms of a -:+ charge ratio (0.5:1, 1:1, 1:1.5, 1:2), the complex having the charge ratio of 1:1 forms a stable polyion complex, which barely shows a reinforcing effect of the R1 relaxivity at a neutral pH. The complexes having the charge ratios of 0.5:1, 1:1.5 and 1:2 fail to form a stable polyion complex due to the imbalance of entire charge of respective polyion complexes, and show R1 relaxivity -reinforcing effect at a neutral pH. However, if a different polymer electrolyte further exists, the R1 relaxivity is further reinforced.

Experimental Example 4:

With regard to a polyion complex of PLL (Gd-DTPA) (16%) and PDEAMA, when the mixing ratio of PLL(Gd-DTPA) (16%) and PDEAMA is varied in terms of a -:+ charge ratio (1:1,1:1.5,1:2,1:4), the complex having the charge ratio of 1:1 forms a stable polyion complex which barely shows a reinforcing effect of the R1 relaxivity at a neutral pH. The complexes having the charge ratios of 1:1.5, 1:2 and 1:4 fail to form a stable polyion complex due to the imbalance of entire charge of respective polyion complexes, and show R1 relaxivity -

reinforcing effect at a neutral pH. However, if a different polymer electrolyte further exists, the R1 relaxing capability is further reinforced.

Experimental Example 5: Acute toxicity of polyion complex of PLL(Gd-DTPA)(16%) and PDEAMA

After adjustment of the final Gd concentration to 2.0 mM with 0.15 M NaCl (pH 7), a contrast agent solution [polyion complex of PLL(Gd-DTPA)(16%) and PDEAMA] containing a mixture of equal amount of [I] [PLL(Gd-DTPA)(16%)] prepared in Example 9 and [II](PDEAMA) prepared in Example 10, was prepared.

This contrast agent solution was intravenously injected to the conscious mice from the tail vein. The mice were monitored for 3 days after the administration, and acute toxicity [LD_{50} (mg/kg body weight)] was estimated. A the result, LD_{50} of polyion complex of PLL(Gd-DTPA)(16%) and PDEAMA was 459 mg/kg body weight.

INDUSTRIAL APPLICABILITY

According to the contrast agent of the present invention, the positive-negative charge becomes imbalanced in the presence of a polymer electrolyte, such as that expressed on an abnormal cell surface, even at a neutral pH (ca. pH 6-8), and a part or the entirety thereof is dissociated due to the substitution phenomenon of the polyion forming the polyion complex or gel, whereby Gd and its surrounding water interact, and the MRI capability is expressed. When the contrast agent do not interact with a polymer electrolyte at a neutral pH, high Gd ions are concentrated within the polyion complex, the polyion gel or the liposome, MRI signal can disappeared because of (i) the T2 effect and/or (ii) inhibition of the diffusion of water molecule from inside to out side of the polyion complex, the polyion gel or the liposome. The MRI contrast agent of the present invention is capable of on-off switching of the MRI capability which reflects the changes in the biological environment, wherein specific polymer electrolyte is expressed due to the occurrence of abnormal cells such as tumor

and the like.

Further, the positive-negative charge in the gels of the present invention becomes imbalanced at an acidic pH (ca. pH 4-6) or an alkaline pH (ca. pH 8-9) in an abnormal cell, then a part or the entirety thereof is dissociated due to the substitution phenomenon of the polyion forming the polyion gel whereby Gd and its surrounding water interact, and the MRI capability is expressed.

In addition, the polyion membrane in the liposome is unstably formed at acidic pH (ca. pH 4-6) or alkaline pH (ca. pH 8-9) in an abnormal cell whereby activated water molecules surrounding Gd in liposome can diffuse to outside of liposome, and the MRI capability is expressed.

CLAIMS

1. An MRI contrast agent, which comprises (i) a complex or a gel of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer being capable of forming a polyion complex or a polyion gel, (ii) a complex or a gel of a polycationic gadolinium (Gd) type contrast agent and an anionic polymer being capable of forming a polyion complex or a polyion gel, or (iii) a liposome containing a metal complex which complexes gadolinium (Gd) with a chelating agent being capable of forming a polyion membrane in the liposome; and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte.

2. The MRI contrast agent of claim 1, which comprises (i) a complex of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer, or (ii) a complex of a polycationic gadolinium (Gd) type contrast agent and an anionic polymer, both complexes being capable of forming a polyion complex, and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte.

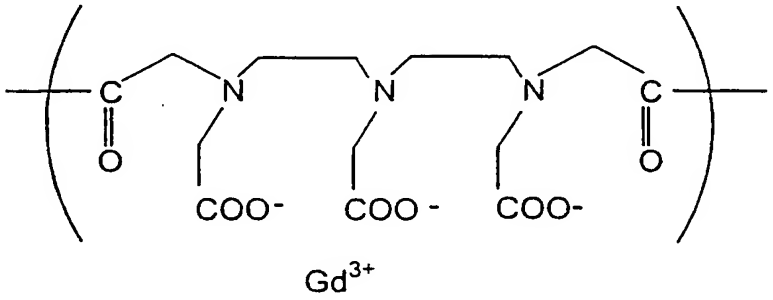
3. The MRI contrast agent of claim 1 which comprises a complex of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer being capable of forming a polyion complex, and which expresses an MRI capability at neutral pH in the presence of a polymer electrolyte.

4. The MRI contrast agent of any of claims 1 to 3, wherein the polyanionic gadolinium (Gd) type contrast agent is (1) a copolymer of (i) a cationic polymer and (ii) a metal complex which complexes gadolinium (Gd) with a chelating agent, and the chelating agent free of gadolinium (Gd) ion, wherein all cations of the cationic polymer are bonded with the metal complex or the chelating agent, or (2) a copolymer of (i) a cationic polymer and (ii) a metal complex which complexes gadolinium (Gd) with a chelating agent.

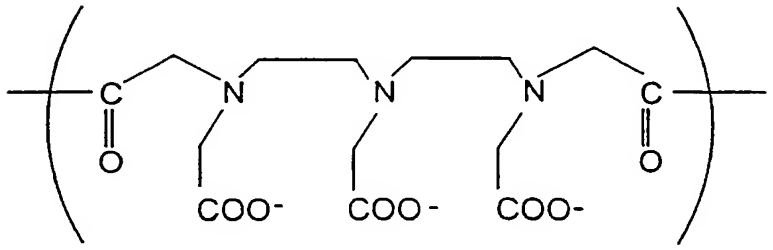
5. The MRI contrast agent of claim 4, wherein the cationic polymer

copolymerized with the metal complex or chelating agent is a natural polymer chitosan and/or at least one synthetic polymer selected from the group consisting of polydiethylaminoethylmethacrylate (PDEAMA), poly•L-lysine (PLL), poly L-histidine (PLH), poly(vinylamine), poly(ethyleneimine), I,m-ionene, poly(N-alkyl-4-vinylpyridinium chloride) (QPVP), poly(vinylbenzyl trimethylammonium chloride) (PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide].

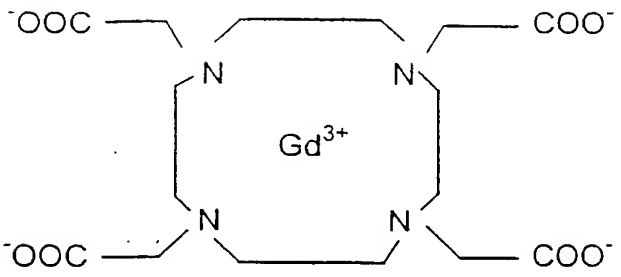
6. The MRI contrast agent of claim 4, wherein the metal complex is a compound partially having the formula



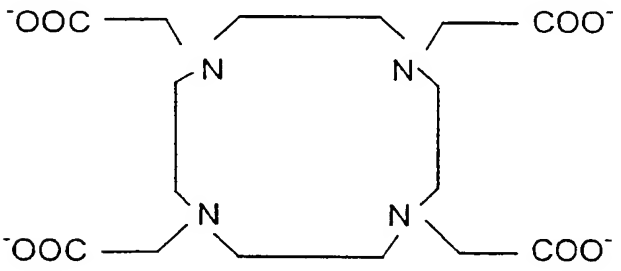
and the chelating agent is a compound partially having the formula



7. The MRI contrast agent of claim 4, wherein the metal complex is a compound partially having the formula,



and the chelating agent is a compound partially having the formula



8. The MRI contrast agent of any of claims 4, 6 and 7, wherein the cationic polymer is poly L-lysine (PLL).

9. The MRI contrast agent of claim 1, wherein the polyanionic gadolinium (Gd) type contrast agent is a polymer contrast agent comprising anionic metal complexes polymerized via a spacer molecule.

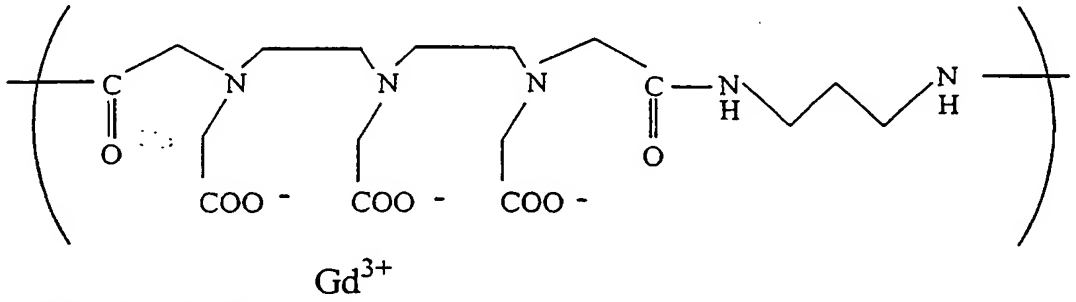
10. The MRI contrast agent of claim 9, wherein the polymer contrast agent comprising anionic metal complexes polymerized via a spacer molecule is a complex polymer of the formula (1)

$$—(Gd—DTPA—PDA)_{x1}—(DTPA—PDA)_{(100-x1)}— \quad (1)$$

wherein DTPA is diethylenetriamine pentaacetic acid, PDA is 1,3-propanediamine, x_1 is a real number of 1 to 99, and the formula

$$—(Gd—DTPA—PDA)—$$

therein shows a DTPA-PDA moiety into which gadolinium (Gd) has been introduced, namely, the complex polymer represented by the formula

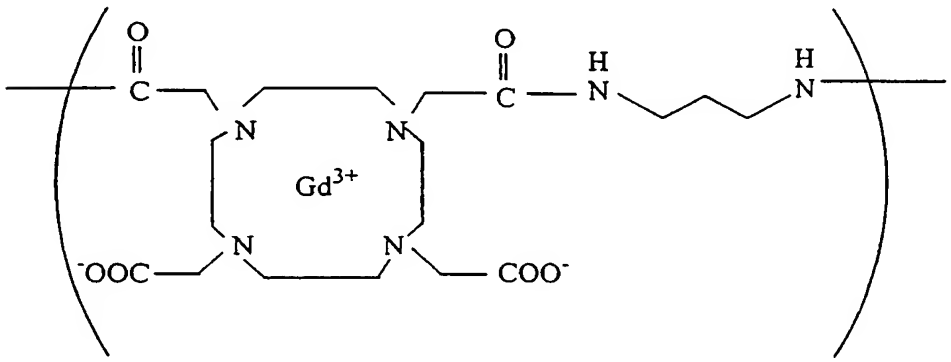


or the formula (2)

(2)

wherein PDA is as defined above, DOTA is 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid, x_2 is a real number of 1 to 49, and the formula

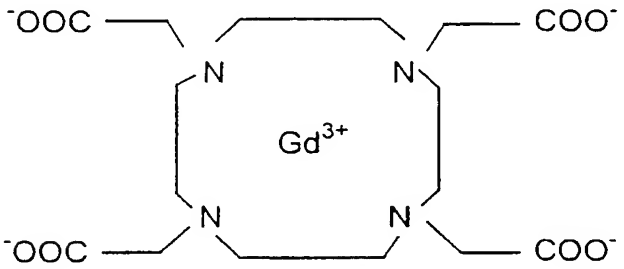
therein shows a DOTA-PDA moiety into which gadolinium (Gd) has been introduced, namely, the complex polymer represented by the formula



11. The MRI contrast agent of claim 1, wherein the cationic polymer is a natural polymer chitosan and/or at least one synthetic polymer selected from the group consisting of polydiethylaminoethylmethacrylate (PDEAMA), poly L-lysine (PLL), poly L-histidine (PLH), poly(vinylamine), poly(ethyleneimine), I,m-ionene,poly(N-alkyl-4-vinylpyridinium chloride) (QPVP), poly(vinylbenzyl trimethylammonium

chloride) (PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide].

12. The MRI contrast agent of claim 1, wherein the polycationic gadolinium (Gd) type contrast agent is a bonded compound of a cationic polymer and a metal complex (Gd-DOTA) of one partially having the formula



wherein the metal complex (Gd-DOTA) has bonded to a part of the cation of the cationic polymer and a part of the cation remains unbonded.

13. The MRI contrast agent of claim 12, wherein the cationic polymer that bonds to the metal complex (Gd-DOTA) is a natural polymer chitosan and/or at least one synthetic polymer selected from the group consisting of polydiethylaminoethylmethacrylate (PDEAMA), poly L-lysine (PLL), poly L-histidine (PLH), poly(vinylamine), poly(ethyleneimine), I,m-ionene, poly(N-alkyl-4-vinylpyridinium chloride) (QPVP), poly(vinylbenzyl trimethylammonium chloride (PVBMA), poly[(N,N-dimethylamino)ethyl acrylate], poly[(N,N-dimethylamino)propyl acrylamide], poly(N,N-dimethylacrylamide), poly(N,N-diethylacrylamide), poly(allylamine), poly[N,N-(dimethylamino)ethyl methacrylate], poly[N,N-(diethylamino)ethyl methacrylate], poly[N,N-(dipropylamino)ethyl methacrylate], poly[N,N-(diethyl)acrylamide] and poly[N,N-(dimethyl)acrylamide].

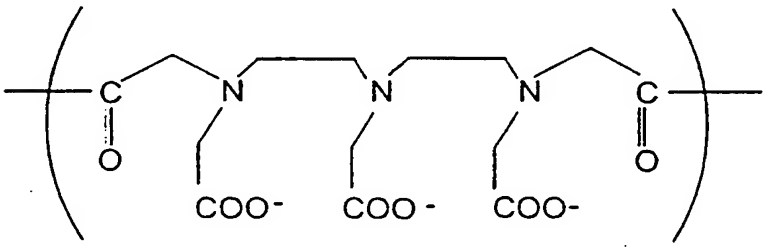
14. The MRI contrast agent of Claim 12, wherein the cationic polymer is poly L-

lysine (PLL) or chitosan.

15. The MRI contrast agent of claim 1, wherein the anionic polymer is at least one member selected from the group of synthetic polymers consisting of poly L-glutamic acid, poly L-aspartic acid, poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly(vinylsulfonic acid), poly(styrenesulfonic acid) (PSS), poly(styrenephosphoric acid) (PSP), polyphosphoric acid, and acidic polysaccharides having colominic acid, sulfonic acid group, carboxylic acid group and/or phosphoric acid group; and the group of natural polymers consisting of hyaluronic acid, chondroitin, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate, and acidic polysaccharides containing sialic Lewis acid, colominic acid, uronic acid, sulfonic acid group, carboxylic acid group and/or a phosphoric acid group.

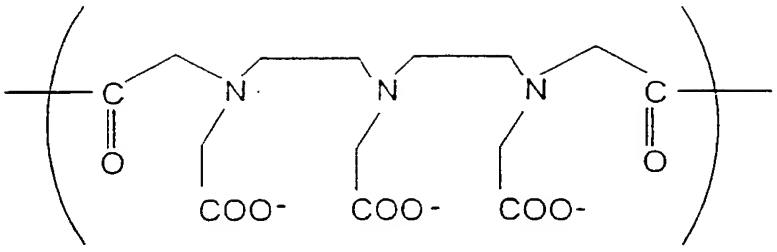
16. The MRI contrast agent of claim 1, wherein the polymer electrolyte is at least one member selected from the group consisting of acid glycolipids and glycosaminoglucans.

17. The MRI contrast agent of claim 1, wherein the polyion complex is a complex of (i) a polyanionic gadolinium (Gd) type contrast agent that is a copolymer of (1) poly L-lysine (PLL) and (2) a metal complex partially having the



formula

and a chelating agent partially having the formula



and

(ii) a cationic polymer that is polydiethylaminoethylmethacrylate (PDEAMA).

18. An MRI contrast agent, which comprises (i) a gel of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer being capable of forming a polyion gel, or (ii) a gel of a polycationic gadolinium (Gd) type contrast agent and an anionic polymer being capable of forming a polyion gel, and which expresses an MRI capability at acidic pH or alkaline pH.

19. An MRI contrast agent, which comprises a liposome containing a metal complex which complexes gadolinium (Gd) with a chelating agent being capable of forming a polyion membrane in the liposome, and which expresses an MRI capability at acidic pH or alkaline pH.

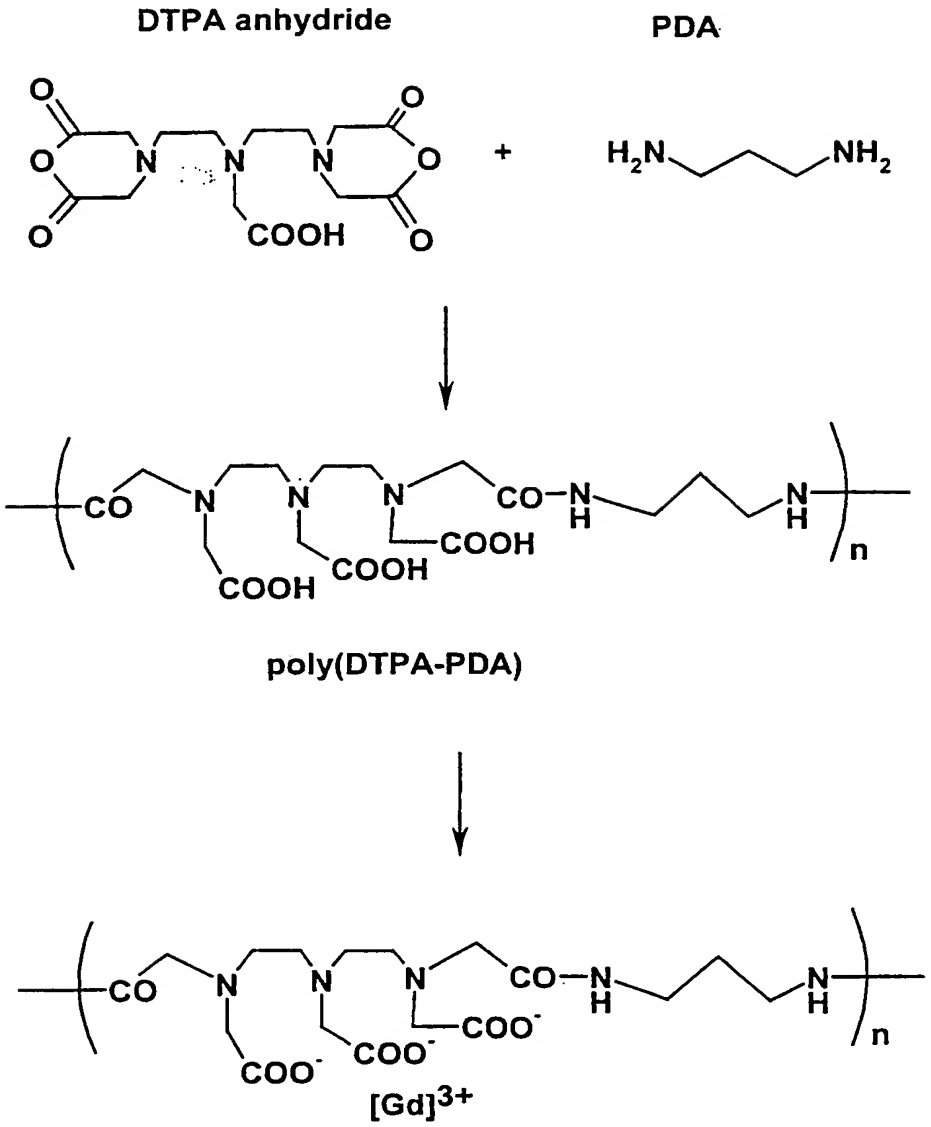


Fig. 1

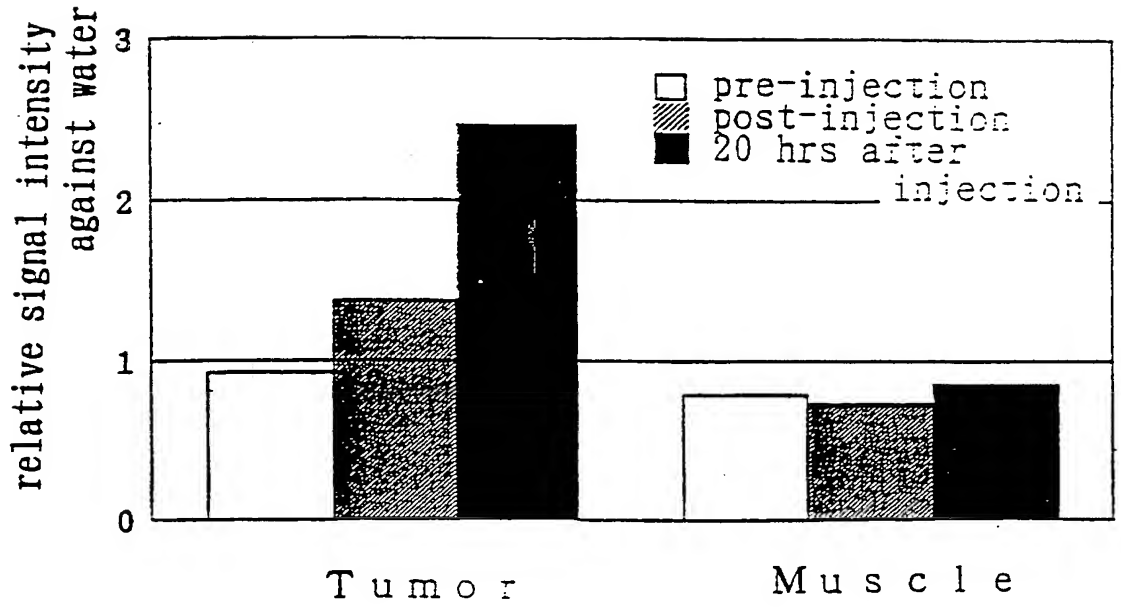


Fig. 2

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Published
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(54) Title: MRI CONTRAST AGENT

(57) Abstract

Provision of an MRI contrast agent wherein imaging capability is expressed only within the target abnormal cells, such as tumor, and imaging is not conducted at the site where imaging is not necessary, thereby to strikingly improve the detection sensitivity of the abnormal cells such as tumor. An MRI contrast agent, which comprises a complex of a polyanionic gadolinium (Gd) type contrast agent and a cationic polymer, or a complex of a polycationic Gd type contrast agent and an anionic polymer, both complexes being capable of forming a polyion complex, and which expresses an MRI capability at a neutral pH in the presence of a polymer electrolyte.

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 99/07090

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MIKAWA, MASAHITO ET AL: "A pH-sensitive contrast agent for functional magnetic resonance imaging (MRI)" CHEM. LETT. (1998), (7), 693-694, July 1998 (1998-07), XP002134101	1-6,8-11
Y	the whole document	1-18
L,P, X	WO 98 41241 A (MIKAWA MASATO ;MIWA NAOTO (JP); AKAIKE TOSHIHIRO (JP); MARUYAMA AT) 24 September 1998 (1998-09-24) * L= priority * the whole document	1-12,17, 18

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2000	- 1. 09. 00

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
Y	US 5 679 810 A (HIMMELSBACH RICHARD J ET AL) 21 October 1997 (1997-10-21) the whole document column 11-12 column 16, line 54-57 column 18, line 29-38; claims; examples	1-18
Y	WO 96 40274 A (NYCOMED IMAGING AS ;COCKBAIN JULIAN (GB)) 19 December 1996 (1996-12-19) claims; examples 11,12	1-18
Y	US 5 554 748 A (WATSON ALAN D ET AL) 10 September 1996 (1996-09-10) the whole document	1-18
Y	WO 96 10359 A (UNIV PENNSYLVANIA ;LEXA FRANK L (US); KACHUR ALEXANDER V (US); ROS) 11 April 1996 (1996-04-11) the whole document	1-18
Y	EP 0 707 857 A (NIHON MEDIPHYSICS CO LTD) 24 April 1996 (1996-04-24) the whole document	1-18
A	WO 98 31712 A (BERNKOP SCHNUERCH ANDREAS ;PAIKL CHRISTINA (AT); GOODRICH CO B F () 23 July 1998 (1998-07-23) the whole document	1-15,17, 18
X	TRUBETSKOY V S ET AL: "NEW APPROACHES IN THE CHEMICAL DESIGN OG GD-CONTAINING LIPOSOMES FOR USE IN MAGNETIC RESONANCE IMAGING OF LYMPH NODES" JOURNAL OF LIPOSOME RESEARCH,US,MARCEL DEKKER, NEW YORK, vol. 4, no. 2, 1 January 1994 (1994-01-01), pages 961-980, XP000619021 ISSN: 0898-2104 figures 1,2	1,16,19
X	US 5 534 241 A (TRUBETSKOY VLADIMIR S ET AL) 9 July 1996 (1996-07-09) the whole document	1,16,19
A	WO 94 03155 A (GEN HOSPITAL CORP) 17 February 1994 (1994-02-17) the whole document	18
A	FR 2 667 072 A (BIOETICA SA) 27 March 1992 (1992-03-27) the whole document	19

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 99/07090

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DE 37 24 985 A (UNIV RAMOT) 11 February 1988 (1988-02-11) the whole document	19

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/07090

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,2,16,18 (partial); 3-11,17, (complete).

   An MRI contrast agent comprising a complex or a gel of a polyanionic gadolinium contrast agent and a cationic polymer being capable of forming a polyion complex or a polyion gel, and expressing MRI capability at neutral pH in the presence of a polymer electrolyte.

2. Claims: 1,2,16,18 (partial); 12-15 (complete).

   An MRI contrast agent comprising a complex or a gel of a polycationic gadolinium contrast agent and an anionic polymer being capable of forming a polyion complex or a polyion gel, and expressing MRI capability at neutral pH in the presence of a polymer electrolyte.

3. Claims: 1,16 (partial), 19 (complete).

   A liposome comprising a chelating agent complexing gadolinium to form a metal complex and being capable of forming a polyion membrane in the liposome, and expressing MRI capability at neutral pH in the presence of a polymer electrolyte.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-19 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Moreover, part iii) of claim 1 relating to the third invention, and claim 19 reciting : " An MRI contrast agent, which comprises a liposome containing a metal complex which complexes gadolinium (Gd) with a chelating agent being capable of forming a polyion membrane in the liposome...." are obscure, not defining in a clear and unambiguous way the subject matter for which protection is sought and are not supported by the description.
Consequently, the search for the three inventions of the present application has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds mentioned in the examples, with due regard to the general idea underlying the application.

Claims searched incompletely: 1-19

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

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